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14. ABSTRACT

The human genome actively transcribes a large amount of non-coding genes, including microRNA and long non-coding RNAs (lncRNAs). Compared to well-known microRNAs, lncRNAs are poorly characterized. However, emerging evidence suggests that lncRNAs may be dysregulated in prostate cancer, but their role in prostate cancer remains elusive. We hypothesize that prostate cancer may exploit this mechanism to promote tumor progression and metastasis, and therapy resistance. Thus, overall goal of this application is to determine whether lncRNAs function as competitive endogenous RNAs (CeRNAs) which are capable of sequestering microRNAs to regulate RNA transcripts. A better understanding of how microRNAs are dysregulated in prostate cancer may help biomarker discovery, ultimately developing a better strategy for prostate cancer treatment.

15. SUBJECT TERMS
microRNA, lncRNA, biomarker

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Introduction

In addition to protein-coding genes, long non-coding RNAs (lncRNAs) may regulate gene expression through various mechanisms. One of them is that lncRNas may function as an endogenous “sponge” and downregulate a series of microRNAs. Thus, lncRNAs have also joined the “competitive endogenous RNA (CeRNA)” regulatory system where microRNA response elements (MREs) may serve as letters of a new language through which microRNAs may regulate not only protein-coding genes, but also non-coding genes. Therefore, the major goal of this application is to determine whether there is CeRNA regulatory system in prostate cancer. The goal of this study was to determine whether lncRNAs

Body

The importance of lncRNAs in human diseases may have to do with their ability to regulate gene expression. Moreover, a major feature for lncRNAs is their ability to interact with either protein, DNA or RNA to exert their functions. For example, lncRNAs may exert their functions through interaction with regulatory proteins such as those chromatin remodeling proteins. Therefore, we searched for potential interactions with microRNAs because emerging evidence suggests that non-coding RNAs may participate in “competitive endogenous RNAs” regulatory network.

The following are our findings based on proposed tasks.

Task 1. Determine the relationship between lncRNAs and microRNAs in prostate cancer and Task 2. Determine the possible reciprocal regulation between them

We identified two lncRNAs, GAS5 and linc-RoR, as potential targets for miR-21 and miR-145, respectively. GAS5 is a 651 nucleotide long RNA consisting of 12 exons (Fig. 1A). A potential miR-21 binding site is located at exon 4 of GAS. Of interest, when we suppressed miR-21 by anti-miR21, we found that it increased

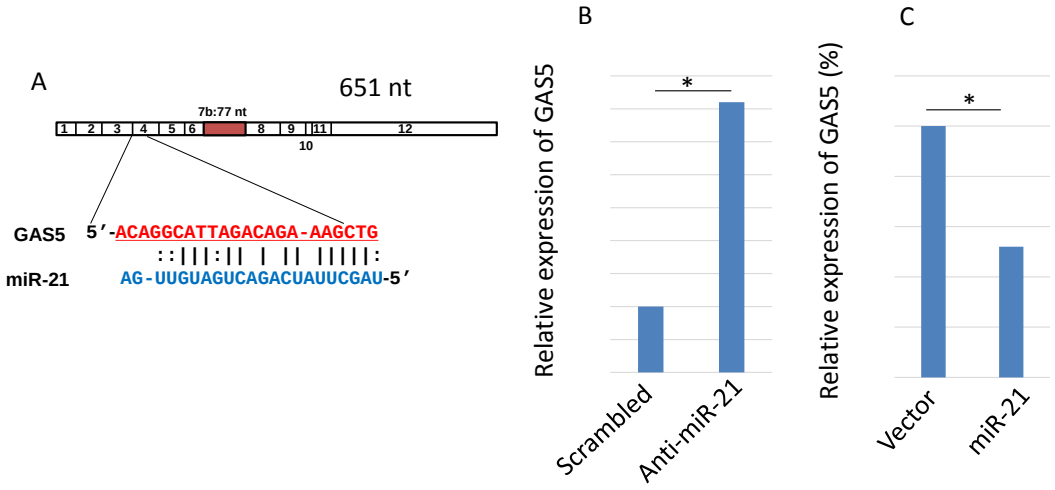

Fig. 1 Identification of GAS5 as a miR-21 target. A, a potential miR-21 binding site in GAS5. B, Anti-miR-21 increases GAS5 expression. C, Ectopic expression of miR-21 suppresses GAS5.

GAS5 expression (Fig. 1B). In contrast, ectopic expression of miR-21 suppressed GAS5 expression (Fig. 1C). Since GAS5 has been implicated as a tumor suppressor and miR-21 as an oncogene, this negative relationship between GAS5 and miR-21 provide an explanation as to why GAS5 is often downregulated whereas miR-21 is often upregulated in cancer.

Similarly, we found two potential binding sites for miR-145 in RoR exon 4 (Fig. 2). RoR was originally identified to be involved in regulation of stem cells. Since RoR is upregulated in prostate cancer, it may function as an oncogene. On the other hand, our previous work suggests that miR-145 is a tumor suppressor. We showed that RoR and miR-145 are co-localized to the cytoplasm, suggesting that they can negatively regulate each other, i.e., a reciprocal negative relationship.

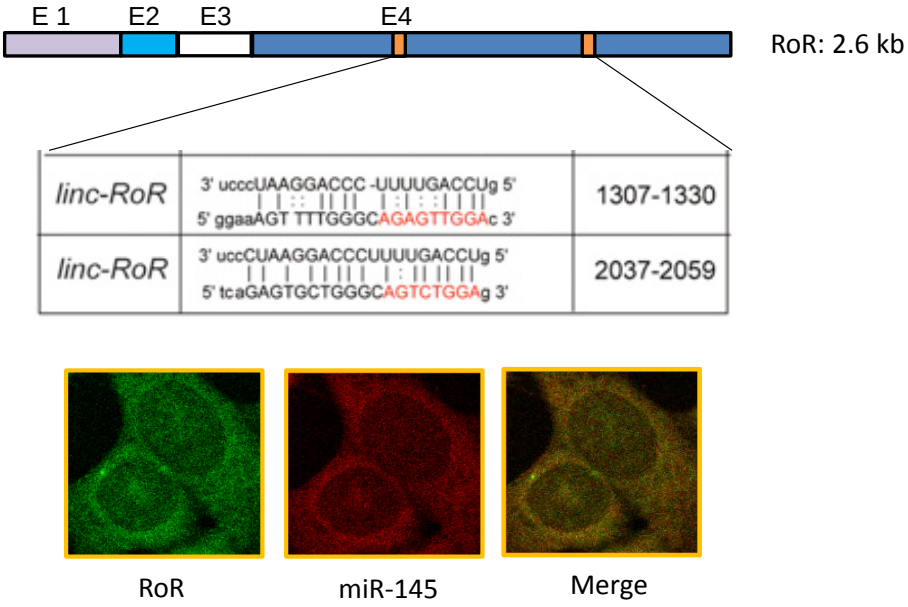

Fig. 2 RoR carries two miR-145 binding sites. Co-localization of RoR (green) and miR-145 (red) predominantly in the cytoplasm by fluorescent in situ hybridization.

Task 3. Identify potential lncRNAs as markers for prostate cancer

In this regard, we found that lncRNA PCGEM1 may serve as a prognosis marker to predict castration resistance. It is well known that androgen deprivation has been the frontline therapy for treatment of advanced prostate cancer. However, the effectiveness of androgen deprivation therapy can only last for a relatively short time and these prostate tumors inevitably become resistant and metastasized. The underlying mechanism is not fully understood. One of the possible reasons is expression of

constitutively active androgen receptor (AR) splice variants. At least 7 AR variants have been identified to date. Among them, AR3 is one of the major AR splice variants identified in prostate cancer cell lines and human prostate cancer tissue and it greatly contributes to prostate cancer progression and castration resistance. However, little is known how AR splice variants are regulated. Our studies suggest that PCGEM1 is able to confer castration resistance by regulating AR splice variants. In this regard, androgen deprivation can induce PCGEM1 expression (Fig. 3A), and moreover, suppression of PCGEM1 by RNAi reduced AR3 (Fig. 3B)

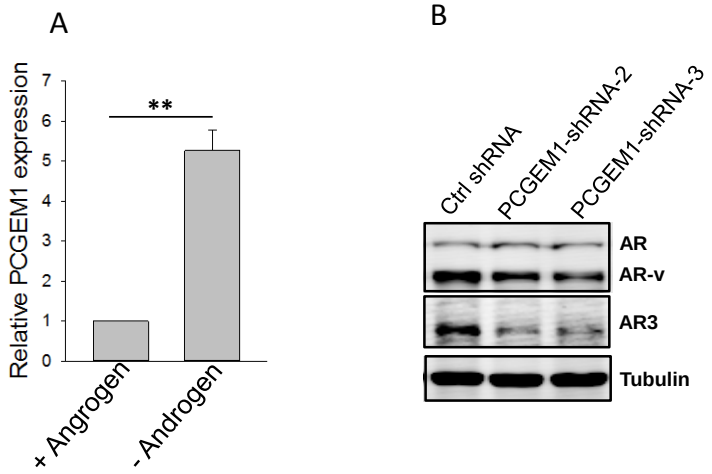

Fig. 3 PCGEM1 promotes castration resistance by regulation of AR3 expression. A, Androgen deprivation induces PCGEM1. B, Suppression of PCGEM1 reduces AR3 expression.

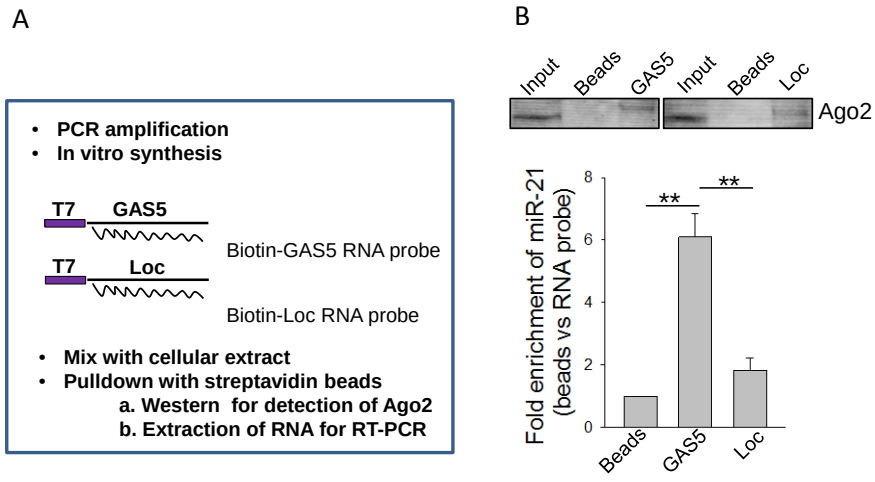

Fig. 4 Identification of the interaction of GAS5 with miR-21 by RNA precipitation. A, A procedure for making biotin-labeled RNA probes and RNA precipitation. B, Detection of GAS5 and miR-21 in the RNA precipitated samples.

Task 4. Determine whether lncRNAs directly interact with miR-21

Since we found that there is a negative correlation between GAS5 and miR-21, and microRNAs silence target genes via RISC complex carrying AGO2, next we asked whether GAS5 directly interacts with miR-12 in the RISC complex. Thus, we synthesized GAS5 RNA probe and labeled with biotin and then mixed with cellular extract (Fig. 4A). After precipitation with streptavidin beads and western blot, we detected AGO2 (Fig. 4B, top right panel), suggesting that GAS5 RNA interacts with AGO2. Importantly, we also detected a significant amount of miR-21 in this GAS5 pulled down pellet carrying AGO2 (Fig. 4B, bottom) while there was only a slight increase in the miR-21 for loc285194 RNA probe (serving as a negative control) (Fig. 4B, bottom). Thus, these results suggest that both GAS5 and miR-21 are likely in the same AGO2 complex and they directly interact each other.

Key Research Accomplishments

- We identified several lncRNAs such as GAS3 and RoR can negatively regulate miR-21 and miR-145, respectively.
- On the other hand, miR-21 and miR-145 can also negatively regulate GAS5 and RoR, respectively. This type of regulation supports a previously proposed "competitive endogenous RNAs (CeRNA)" theory.
- Identification of this reciprocal repression relationship between lncRNAs and microRNAs is important to better understand prostate cancer biology and biomarker discovery.

Reportable Outcomes

A manuscript is in preparation

Conclusions

Our results suggest that there is an CeRNA regulatory system in prostate cancer in which lncRNAs and microRNAs are regulated each other to impact prostate tumorigenesis. Although it is known that microRNAs can silence a variety of coding genes, our results indicate that microRNAs can also target non-coding genes such as lncRNAs. Furthermore, these findings may provide a basis for future to explore whether combination of lncRNAs and corresponding microRNAs as better markers for prostate cancer diagnosis and prognosis.